DRY SKIN PREPARATIONS OF FROGS AND TOADS AS AIDS FOR THEIR TAXONOMY¹

The paper describes the modified method of preparation of anuran dry skins. It also discusses in detail, for the first time, utility of the dry skin preparations as an additional, important, convenient and very useful means of amphibian taxonomy. For the purposes of discussion, black and white photographs of dry skin preparation of one species each of *Bufo, Microhyla* and *Philautus* and four species of *Rana* have been taken into account. Wherever necessary differences in the male and female of a species have also been presented.

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INTRODUCTION

The method developed by Kincaid (1948) for preserving the colour pattern of the skins of frogs is very useful (Knudsen 1966). It appears to have been used by both of them solely from the point of view of colour preservation.

It is known that, besides the skin colour, the nature of the skin and its marking patterns, the nature of fingers and toes, the extent of webbing, separation of metatarsals by the web and nature of the metatarsal tubercles happen to be some of the characters of taxonomic importance in the anuran classification. These characters, being essentially external ones, are far more valuable than other internal characters, in visual indentification of the species during field work (Paranjape and Mulherkar 1979).

From this point of view, the earlier 'skin technique' has been modified to a certain ex-

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MATERIAL AND METHOD

To prepar an album of dry skins the following procedure is followed:

The frog (or toad) is etherised. It is then quickly skinned by taking incision mid-ventrally along the body and carrying it on to the limbs. The skin along the jaws and on the head region is separated from the body, as it is somewhat firmly attached. Elsewhere, the skin being loosely attached to the body presents very little difficulty in its separation. The incisions along the limbs are continued upto $\frac{1}{2}$ or $\frac{3}{4}$ of the palm and the sole regions. Thereafter, the skin on the digits (i.e. fingers and toes) is removed by gently pulling of the separated skin, in the manner similar to

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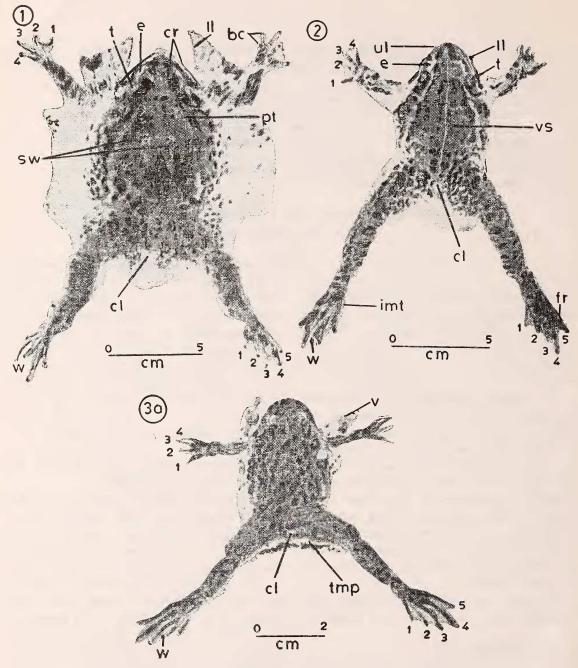


Fig. 1. Bufo melanostictus Schneider; Common Indian Toad (Fam. Bufonidae, Genus Bufo). Fig. 2. Rana tigerina Daud. Indian Bull Frog (Fam. Ranidae, Genus Rana, Subgenus Rana). Fig. 3a. Rana cyanophlyctis Schneider. Skipper Frog (Fam. Ranidae, Genus Rana, Subgenus Rana). A male.

Abberviations: bc, black cornifications; cl, cloacal aperture; cr, cranial ridges; e, eye; fr, fringe; imt, inner-metatarsal tubercle; 11, lower lip; sw, two series of warts; t, tympanum; tmp, typical marking pattern; ul, upper lip; v, vocal sac; vs, vertebral streak; w, web. the removal of a sock. The entire skin is then spread by floating it in water. The everted skin on the digits needs either very careful turning outside in or extending of the incision to each digit. With the support of the phalanges thus lost, this region presents difficulty in the spreading. The extension of the web along the digits, which has taxonomic importance (Boulenger 1920; Daniel 1963), is also not effectively spread.

In order to overcome these difficulties and depending upon the size of the animal, metacarpals/metatarsals were either partly or wholly retained together with the phalanges, by adjusting the extent of the incisions in the palm/ sole regions. For example in large sized specimens they were partly, proximally cut. In small and delicate specimens these bony structures were entirely retained. It has been observed that retention of the phalanges not only facilitates proper spreading of the web, but also preserves the nature of the digit and its tip. Similarly by retaining the metatarsals the extent of separation of the outer metatarsals can be studied. Alongwith these characters careful retention of the vocal sacs, outer and inner metatarsal tubercles, when present, aids further in enhancing the value of the dry skin preparation from the taxonomic point of view. Thus Kincaid's method is modified by us (Paranjape and Mulherkar 1977).

The rest of the procedure regarding spreading, mounting, preservation of the dry skin preparation etc., was mostly followed as described by Knudsen (1966). However, the shape of the trunk region is particular in certain families of frogs. The trunk is rather short, much broad in the middle and shows a sudden posterior narrowing in frogs belonging to Microhylidae and Rhacophoridae. With the usual mode of spreading (Figs. 1-4 & 6-8) it was noticed that the typical shape is not clearly visible. Therefore the mode of spreading was also modified in the case of a microhylid frog (Fig. 5). In this preparation the limbs were so positioned as to simulate their natural arrangement as far as possible. The result was found to be encouraging and to this extent also the earlier method has been modified by us.

The modified method besides retaining the skin-colour, ensures retention of other external characters of taxonomic importance as can be seen from the discussion.

DISCUSSION

Various external characters that are retained in the modified method enable in classifying anuran amphibia. Some specific-examples are as follows:

1. Nature of the skin: In this character, whether the skin is warty, tuberculate, granular or smooth is taken into account. For example, the skin is distinctly warty or heavily tubereculate in toads (Fig. 1). It is generally smooth (Fig. 2) or granular in frogs. (Most of the frogs belonging to the genus Rana dorsally bear longitudinal skin-folds or ridges. They are of varying lengths and give a characteristic wrinkled appearance. Due to flattening and pressing this character cannot be retained in the dry skin preparations). Further observation of the skin of toad indicates presence of a pair of large parotoid glands (Fig. 1) that are always present in most of the toads (absent in the genus Ansonia) and absent in frogs. Further, the presence of two series of heavy, roundish, black-tipped warts, prominent cranial ridges, tips of fingers and toes and tubercles on palm crowned with dark black cornifications indicate that the toad-skin is of an adult of Bufo melanostictus (Fig. 1). It can be noted here that these characteristic black

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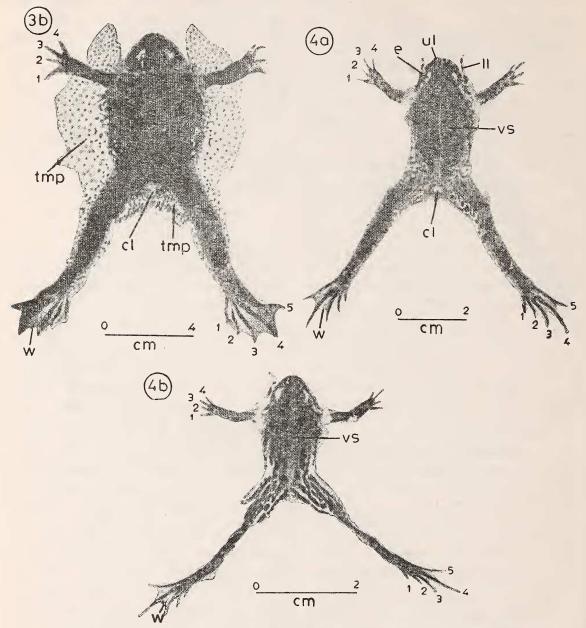


Fig. 3b. Rana cyanophlyctis Schneider. Skipper Frog. A female. Fig. 4a & 4b. Rana limnocharis Weign. Indian Cricket Frog or Grass Frog (Fam. Ranidae, Genus Rana, Subgenus Rana).

Abbreviations: cl, cloacal aperture; c, eye; tmp, typical marking pattern; ul, upper lip; vs, vertebral streak; w, web. cornifications are peeled off and thus lost in , preserved specimens (Daniel 1963). They are, however, nicely retained in the preparation mentioned above.

2. Colour and marking patterns: These are also of taxonomic importance which are seen in actual dry skin preparations. However, in black and white photographic reproduction provided here, only the marking pattern can be described here. Frog's skin is invariably variously marked with spots, bars, patches on the dorsal side. They show considerable size and pattern variation in the different species. Ventrally the skin is creamy or yellowish white and at times mottled or stippled. From the nature of spots one can readily distinguish three species of the genus Rana, namely, R. tigerina (Fig. 2) from R. cyanophlyctis (Fig. 3) or from R. limnocharis (Fig. 4). Further it can be seen that spots in the form of transverse bands are practically spread across the hind-limbs in R. tigerina while they are not band-like and do not reach across the hindlimbs in R. cyanophlyctis and R. limnocharis. By colour and marking pattern the male and female of a species can also be identified. For example, the male of R. cyanophlyctis (besides its small size and bluish vocal sacs) bears a white band with dark edges on the back of the thighs (Fig. 3a). Although white spots are present in the female of this species a continuous band is wanting in it. Similarly skin of the female of *R. cyanophlyctis* ventrally shows a dotted appearance (Fig. 3b), especially so, during the breeding season. The male lacks such an appearance (Fig. 3a). Sometimes the marking pattern is so typical of a species that a mere look enables one to visually identify it reasonably correctly, in the field. For example, there is a characteristic blackishbrown mark that begins between the eyes and touching them. It extends posteriorly, shows

deep emarginations as it broadens and finally it spreads over the thighs in the form of two stripes. It is flanked by relatively uniform greyish coloration. This is characteristic of *Microhyla ornata* (Fig. 5). A rhacophorid frog, *Philautus bombayensis*, shows considerable colour variation but generally shows a dice-box shaped mark on the dorsal side of the trunk. It has a faint coloured band-like mark slightly constricted in middle, broad at the ends and it is flanked by dark coloration (Figs. 6, 7). In routine preservation not only the colour but this marking pattern also gencrally fades away.

Presence of certain marks also helps in identification. For example, there is seen in some forms a V shaped mark extending between the eyes. It is narrow, acutely pointed and more V-like in *R. limnocharis* (Fig. 4a) while broad, obtusely pointed in *P. bombay*ensis (Fig. 7). Similarly marking pattern of lips is also useful. For example, in *R. tigerina* (Fig. 2) the upper lips shows a light canthal streak with blackish elongated spots and the lower shows large black spots. In *R. limno*charis the lips bear dark bars (Fig. 4a).

The mid-dorsal vertebral streak is yet another marking pattern that aids in identification. It may be absent altogether, as is the case in R. cyanophlyctis (Fig. 3) or may be present. If present it may be complete, that is extending from the snout to vent or may be incomplete, that is reaching the vent but not the snout. Similarly, it may be narrow or broad. The streak can also be of different coloration. For example, in R. tigerina (Fig. 2) the vertebral streak is narrow, complete and pale yellowish-white in colour. in R. limnocharis (Fig. 4a) it is narrow, generally incomplete and pale yellowish-white in couour. In another type of R. limnocharis (Fig. 4b) it is however, complete, broad and pinkish

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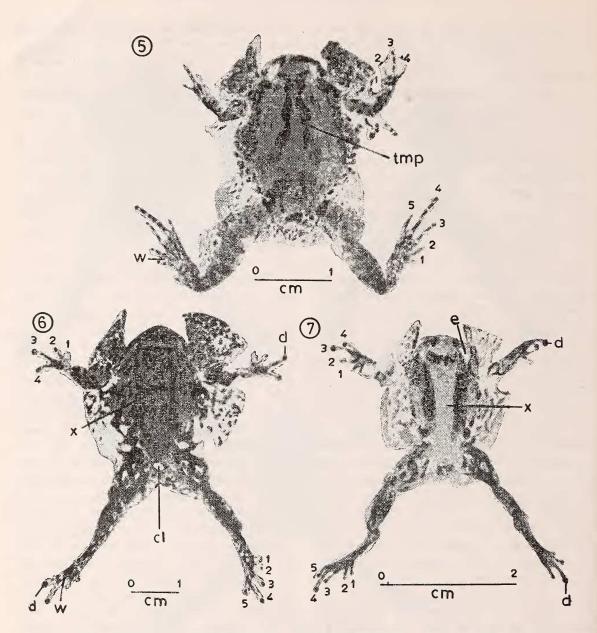


Fig. 5. Microhyla ornata Dum. & Bibr. Narrow-mouthed frog. (Fam. Microhylidae, Genus Microhyla). Figs. 7 & 8. Philautus bombayensis Gunth. (Fam. Rhacophoridae). Abbreviations: cl, cloacal aperture; d, disc; e, eye; tmp, typical marking pattern; w, web; x, area of the disc-boxlike mark.

brown in colour.

3. Fingers: These are also helpful in identification. The tips of the fingers may be with or without discs. They may be long and slender or short and thickly set. The relative lengths of the 1st and 2nd fingers is also a diagnostic character.

For example the fingers (as also the toes) bear somewhat oval adhesive discs in *R. temporalis* (Subgenus: *Hylorana*, genus: *Rana*) (Fig. 8). This is an adaptation for its rockystream dwelling or semi-arboreal habitat. The fingers (as also the toes) in tree-frogs (Fam. Rhacophoridae) possess circular adhesive discs, as can be seen in *P. bombayensis* (Figs. 6 & 7).

The first finger is nearly as long as or a little longer than the second in *R. temporalis, R. limnocharis.* It is generally longer in *R. tigerina,* distinctly so in the Indian burrowing frog, *R. breviceps* (Subgenus: Tomopterna). It is more or less of equal length in *R. cyanophlyctis* and quite short in *M. ornata.* 4. Toes and webbing pattern: Reference has already been made (in 3) whether the tips of the toes are with the discs or not. Other toc-features of relatively minor importance are whether they are long and slender or some-

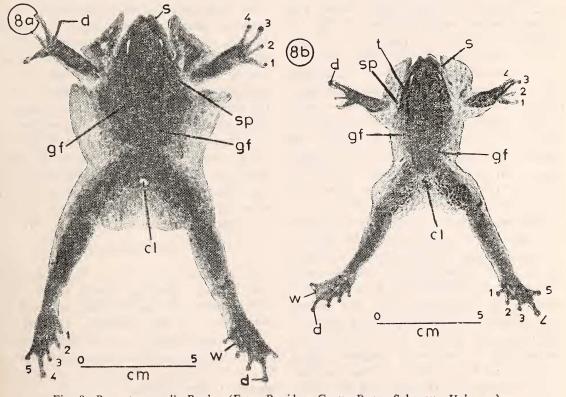


Fig. 8. Rana temporalis Bouln. (Fam. Ranidae, Genus Rana, Subgenus Hylorana). 8a-male, 8b-female. Abbreviations: cl, cloacal aperture; d, disc; gf, glandular fold; s, light streak on lip; sp, spot at the angle of jaws; t, tympanum; w, web.

what stout, whether the tips are somewhat rounded or not.

The extent of webbing forms a very important diagnostic character (Daniel 1963). We feel that in our modified method mentioned earlier, this valuable character is beautifully expressed. For example, the webbing is rudimentary in M. ornata (Fig. 5) and is rather poor in B. melanostictus (Fig. 1) although the degree of webbing is of $\frac{1}{4}$ or of $\frac{1}{2}$ type. In P. bombayensis (Figs. 6, 7) the disced toes are not more than 1/3 webbed. In R. limnocharis (Fig. 4) the toes are generally half webbed with three phalanges of the 4th toe free. In R. temporalis (Fig. 8) the toes are $\frac{3}{4}$ or practically fully webbed and the web typically extends upto the discs of the 3rd and 5th while almost two phalanges of the 4th toe are uncovered by it and hence free. The toes are practically fully webbed in R. tigerina (Fig. 2). However, the web does not reach the tip of the 3rd toe and the 5th toe bears an outer fringe of web. This is a feature of forms that are more aquatic in nature. The degree of webbing is typically of the fullest type in R. cyanophlyctis (Fig. 3) and the web, as it reaches all the toe-tips shows deep emarginations when toes are spread out.

5. Other miscellaneous characters: The extent of attachment or separation of the two external metatarsals (viz., 4th and the 5th) also aids in identification. For example, the outer metatarsals are united in the basal $\frac{1}{2}$ or 1/3 region in *R. limnocharis* (Fig. 4b) or are bound together in *R. breviceps*. They are practically separated upto the base by web in *R. tigerina* (Fig. 2) and in *R. temporalis* (Fig. 8). Glandular folds, streaks, as also the relative size difference in the male and the female are also useful in identification. For example, in *R. temporalis* there are seen two dorso-lateral, glandular folds. Each extending from above the tympanum to cloaca on that side. The two folds enclose between themselves a fairly broad, light-coloured oval patch on the back (Fig. 8b). In the same frog there runs along the border of the upper lip a light, dirtywhitish streak that also posteriorly forms a similar spot near the angle of the jaws (Fig. 8a).

The nature of inner and outer metatarsal tubercles is also useful in identification. For example, the inner metatarsal tubercle is relatively small and obtuse in R. tigerina (Fig. 2) but is large, crescentic and shovel-shaped in R. breviceps. Both inner and outer metatarsal tubercles are present in R. limnocharis and in M. ornata. However, the nature of the tubercles is not well retained when the skin is subjected to pressure in a herbarium press. The character, is therefore, not very satisfactorily retained, in general, in this method.

In frogs, in general, the male is smaller in size as compared to the female of the same species, for example, *R. cyanophlyctis* (Fig. 3a, 3b). However, the male is large and with strong fore-limbs, as compared to the female, for example, *R. temporalis* (Figs. 8a, 8b).

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